

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

Claim 1 (original): A method for producing a nutrient medium for growing mammalian or insect cells in culture whereby for at least one of H, C or N, substantially all atoms in substrates that are used by the cells for synthesis of biomolecules in the nutrient medium are isotopically labelled, whereby the method comprising the steps of:

(a) growing an organism on a mineral medium which supports growth of the organism, whereby in the medium substantially all of the assimilable atoms, for at least one of H, C or N, are isotopically labelled, to produce labelled biomass;

(b) autolysing the biomass of an organism grown as in (a) to produce an autolysate;
and,

(c) composing the nutrient medium by combining the autolysate as obtained in (b) with further components necessary for growth of the mammalian or insect cells.

Claim 2 (original): A method according to claim 1, wherein the organism is a fungus, yeast or algae.

Claim 3 (original): A method according to claim 2, wherein the organism is an organism that belongs to a genus selected from Saccharomyces, Pichia, Hansenula,

Kluyveroznyces, Candida, Brettanomyces, Debaryomyces, Torulopsis, Yarrowia, Galdieria, Cyanidiunt, Porphyridiunt, Cystocloniunt, Audouinella, and Cyanidioschyzon.

Claim 4 (currently amended): A method according to ~~any one of claims 1-3~~claim 1, wherein the method further comprises the steps of:

(a) growing an organism on a mineral medium which supports growth of the organism, whereby in the medium substantially all of the assimilable atoms, for at least one of H, C or N, are isotopically labelled, to produce labelled biomass;

(b) extracting biomass of an organism with an organic solvent to produce an extract comprising lipids, whereby the organism is grown as in (a) or is grown as in (a) on a medium without isotopic substitution;

(c) hydrolysing biomass of an organism grown as in (a) at a non-alkaline pH to produce a hydrolysate comprising amino acids;

(d) composing the nutrient medium by combining the autolysate as obtained in any one of claims 1 - 3 with amino acids as obtained in (c) and adding further components necessary for growth of the mammalian or insect cells.

Claim 5 (currently amended): A methods according to claim 4, wherein in step (d) the nutrient medium is composed by combining the autolysate ~~obtained in any one of claims 1-3~~ with the amino acids ~~obtained in (c)~~ and the lipids ~~obtained in (b)~~ and adding further components necessary for growth of the mammalian or insect cells.

Claim 6 (currently amended): A method according to ~~any one of claim 1—5~~ claim 1, whereby the nutrient medium is composed of autolysate, lipids and amino acids obtained from at least two different organisms.

Claim 7 (currently amended): A method according to ~~any one of claims 1—6~~ claim 1, whereby, prior to hydrolysis in (c), lipids and pigments are extracted from the biomass using an organic solvent.

Claim 8 (currently amended): A method according to ~~any one of claims 1—7~~ claim 1, whereby the organism from which the lipids are extracted, belongs to a genus selected from the group consisting of Rhodophyta, Cyanidiophyceae, Chlorophyta, Cyanophyta, Diatoms, Phaeophyceae, Dinoflagellate, Dinophyta and Galdieria.

Claim 9 (currently amended): A method according to ~~any one of claims 1—8~~ claim 1, whereby the organism from which the hydrolysate comprising amino acids is produced, is an organism selected from the group consisting of algae, fungi, yeasts and methylotrophic bacteria.

Claim 10 (original) A., method according to claim 9, whereby the organism belongs to a genus selected from the group consisting of Pichia, Saccharoinyces, Hansenula, Cvanidiunr, Ga/dienia, Porphyndiunr, Spirzdirra, and Merhvlobacilhis.

Claim 11 (currently amended): A method according to ~~any one of claims 1–~~
~~10~~claim 1, whereby the further components necessary for growth of the mammalian or insect
cells comprise one or more of:

- (a) one or more of glucose, fructose, and sucrose;
- (b) one or more Krebs-cycles intermediates selected from the group consisting of citrate, succinate, fumarate, maleic acid, oxalate and malate; (c) pyruvate; and,
- (d) one or more vitamins selected from the group consisting of thiamin, riboflavin, niacin, vitamin B6, folic acid, vitamin B 12, biotin, pantothenic acid, choline, paraaminobenzoic acid and alpha-tocopherol.

Claim 12 (currently amended): A method according to ~~any one of claims 1–~~
~~10~~claim 1, whereby substantially all atoms in substrates that are used by the mammalian or insect
cells for synthesis of biomolecules in the nutrient medium are isotopically labelled with an
isotope selected from ^{15}N ; ^{13}C ; ^2H ; ^{15}N and ^{13}C ; ^{15}N and ^2H ; ^{13}C and ^2H ; or ^{15}N , ^{13}C and ^2H .

Claim 13 (currently amended): A method for producing a biomolecule, whereby
substantially all atoms in the biomolecule are isotopically labelled, the method comprising the
steps of:

(a) growing a culture of mammalian or insect cells capable of producing the biomolecule under conditions conducive to the production of the biomolecule, in a nutrient medium produced in a method according to ~~any one of claims 1—12~~claim 1; and

(b) recovery of the biomolecule.

Claim 14 (original): A method according to claim 13, wherein the biomolecule is a soluble protein or a membrane protein.

Claim 15 (original): A method according to claim 14, wherein the mammalian or insect cells capable of producing the protein comprise an expression vector comprising a nucleotide sequence coding for the protein.

Claim 16 (currently amended): A method according to ~~claims~~claim 14 or 15, wherein the protein is a mammalian protein.

Claim 17 (currently amended): A method for obtaining structural information on a biomolecule, the method comprising the steps of:

(a) producing a biomolecule, whereby substantially all atoms in the biomolecule are isotopically labelled, in a method according to ~~any one of claims 13—15~~;

(b) optionally, purifying the biomolecule;

(c) subjecting the biomolecule to spectroscopic analysis to obtain information about its structure.

Claim 18 (original): A method according to claim 17, wherein the spectroscopic analysis comprises NMR spectroscopy.

Claim 19 (currently amended): A method according to ~~claims~~ claim 17 or 18, wherein the structural information on a biomolecule is information about the three-dimensional structure of the biomolecule.

Claim 20 (currently amended): A method according to ~~any one of claims~~ claim 17-19, wherein the biomolecule is a protein complexed to a second biomolecule.

Claim 21 (currently amended): A method according to claim 20, ~~wherein the second biomolecule is produced in a method according to any one of claims 1-10 and whereby~~ 20 - 100% of the hydrogen atoms in the second biomolecule are uniformly substituted with the isotope ^2H .

Claim 22 (original): A method according to claim 21, wherein the second biomolecule is a protein.

Claim 23 (original): A nutrient medium for the production of an isotopically labelled biomolecule from mammalian or insect cells, the medium supporting growth of a mammalian or insect cell culture under condition conducive to the production of the biomolecule, the medium comprising:

- (a) a mixture of inorganic salts;
- (b) a source of amino acids;
- (c) a carbohydrate energy source;
- (d) a source of lipids;
- (e) optionally, a protective agent;
- (f) optionally, vitamins and/or organic compounds;
- (g) optionally, organic acids; and,
- (h) optionally, trace elements;

whereby substantially all atoms in (a), (b), and (c), and, optionally in (d), (e), (f), (g) and (h) are isotopically labelled for at least one of H, C or N or whereby 20 - 100% of the hydrogen atoms in (a), (b), and (c), and, optionally in (d), (e), (f), (g) and (h) are uniformly substituted with the isotope ^2H .

Claim 24 (original): A nutrient medium according to claim 23, whereby the source of amino acids comprises an hydrolysate comprising amino acids that is produced from yeast biomass, whereby the hydrolysis of the biomass comprises autohydrolysis.

Claim 25 (currently amended): A nutrient medium according to ~~claims-claim 23-or~~
24, whereby the source of lipids comprises fatty acids, steroids, and lipid soluble vitamins.

Claim 26 (currently amended): A nutrient medium according to ~~any one of claims~~
~~23-25~~claim 23, whereby the carbohydrate energy source is one or more of glucose, fructose,
and sucrose; the organic acids are one or more of pyruvate and the Krebs-cycles intermediates
selected from the group consisting of citrate, succinate, fumarate, maleic acid, oxalate and
malate; the vitamins are one or more vitamins selected from the group consisting of thiamin,
riboflavin, niacin, vitamin B6, folic acid, vitamin B12, biotin, pantothenic acid, choline, para-
aminobenzoic acid and alpha-tocopherol.

Claim 27 (currently amended): A nutrient medium according to ~~any one of claims~~
~~23-26~~claim 23, whereby substantially all atoms in (a), (b), and (c), and, optionally in (d), (e),
(f), (g) and (h) are isotopically labelled with an isotope selected from ^{15}N ; ^{13}C ; ^2H ; ^{15}N and ^{13}C ;
 ^{15}N and ^2H ; ^{13}C and ^2H ; or ^{15}N , ^{13}C and ^2H .

Claim 28 (original): A mammalian membrane protein whereby substantially all atoms
in the protein are isotopically labelled with an isotope selected from ^{15}N , ^{13}C ^2H ^{15}N and ^{13}C , ^{15}N
and ^2H , ^{13}C , and ^2H , or ^{13}C and ^2H .

Preliminary Amendment
Based On PCT/NL2004/000503
SUGHRUE MION, PLLC Ref: Q92604

Claim 29 (original): A mammalian membrane protein whereby 20 - 100% of the hydrogen atoms in the protein are uniformly substituted with the isotope ^2H .

Claim 30 (currently amended): A mammalian membrane protein according to ~~claims claim 28 or 29~~, whereby the protein is a human protein.